

[0066] The breeding values of the bulls are centrally estimated by the United Information Systems Animal Production (Vereinigte Informationssysteme Tierhaltung—VIT) in Verden. A total amount of more than 150,000 daughters and their performance data are integrated in the estimation of the breeding values. From all bulls, deregressed breeding values, concerning the milk yield, the protein and fat yield, the protein content (in %) and the fat content (in %), are utilized in the variance component estimation. The deregression of the breeding values is carried out as described by Thomsen et al. (2001, *J Anim Breed Genet.* 118, 357-370).

[0067] The variance component estimation is carried out using the program package SAS. First, as unique fixed effect, the marker CSN1S1 is considered in the model, because other influence factors (e.g. operational effects, milking frequency) are already corrected in the frame of the estimation of the breeding value and the deregression (influence of the sires). The analysis reveals significant effects of the marker CSN1S1 on all studied traits (deregressed breeding values for protein percentage (DRG_PP), milk yield (DRG_MY1), fat yield (DRG_FY1), protein yield (DRG_PY1), fat percentage (DRG_FP)). Table 3 shows the effect of CSN1S1 on deregressed breeding values for milk production traits, indicating also the probability of error (p) for the effects on the individual traits.

TABLE 3

Trait	Probability of error (p)
DRG-PP	<0.0001
DRG_MY1	0.0011
DRG_FY1	0.0016
DRG_PY1	0.0056
DRG_FP	0.0052

[0068] The highest significance is calculated for the effect on DRG_PP. As the examined marker CSN1S1 is located directly within the regulatory region of a milk protein gene,

this could be an indication of a direct effect. The marker CSN1S1 fulfils the requirements to a functional candidate gene.

[0069] The highest breeding value for milk (DRG_MY1) is achieved on average by bulls with the genotype 12, whereas the highest breeding values for protein percentage (DRG_PP) are found within the group with genotype 24. Table 4 shows a compilation of the least square means (LS_means) for the groups with the genotypes 12, 22, 23 and 24. The table displays the LS_means as well as standard errors for the deregressed breeding values for milk yield (DRG_MY1) and protein percentage (DRG_PP) in groups with different CSN1S1 genotypes.

TABLE 4

CSN1S1 type	n	LSMEAN \pm se	
		DRG_MY1	DRG_PP
12	79	198.232 \pm 15.700	-0.00022534 \pm 0.00006470
22	398	155.341 \pm 6.995	-0.00037495 \pm 0.00002921
23	131	138.806 \pm 12.192	-0.00038405 \pm 0.00005271
24	76	112.364 \pm 16.007	0.00008175 \pm 0.00006650
Alle	684	152.353	-0.000307

[0070] In order to obtain a more exact clarification, the variance analysis is repeated within individual families and groups of families with identical genotypes. Hereby is revealed, that the effect on the milk yield can not be confirmed in all families. In family 9, in which the sires exclusively passed down the allele 2, the only remaining effect is encountered close to the 5% threshold of significance for DRG_PP (p=0.0610). Furthermore, a comparison of the LS_means for the traits DRG_MY1, DRG_PP, DRG_FP is carried out for all groups of genotypes and within each individual family, and it is proved whether the difference of the LS-means between the genotypes 12, 23 and 24 and the most frequent genotype 22 is significant. The results are graphically illustrated in FIG. 5.

SEQUENCE LISTING

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<220> FEATURE:
<221> NAME/KEY: alpha-S1Kaseingen
<222> LOCATION: (1)..(1061)
<223> OTHER INFORMATION: start Exon 1 at position 620
<300> PUBLICATION INFORMATION:
<301> AUTHORS: Koczan Dirk, Hobom Gerd, Seyfert Hans-Martin
<302> TITLE: Genomic organization of the bovine alpha S1-casein gene
<303> JOURNAL: Nucleic acids research
<304> VOLUME: 19
<305> ISSUE: 20
<306> PAGES: 5591
<307> DATE: 1991-09-24
<308> DATABASE ACCESSION NUMBER: X59856
<309> DATABASE ENTRY DATE: 1991-07-18
<313> RELEVANT RESIDUES: (1)..(1061)
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gattagacca catataatgt aacttatttc acaaggtaaa taattataat aaataatatg 180
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taaccataaa tctaggggtt tgttgggggtt tttttttggt tgtaattta gaacaatgcc 420
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agagctatag gtgaaaaatt aaatactttt ataaagacca aattgatcat ttttaaacga 900
aattcttata tactgaaaat gtagatacat aacttcagta tagattttatg gtaaaataat 960
ttgaatcatt tttgtcaaat tctgtaaaaa gttgtcatat agaataattt ataataattt 1020
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gattagacca catataatgt aacttatttc acaaggtaaa taattataat aaataatatg    180
gattaactga gttttaaaag gtgaaataaa taatgaattc ttctcatggt cttgtatggt    240
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tgaatttttc aaaggttaca aaggaaattt tattgatata ataatgcat gttctcataa    360
taaccataaa tctagggttt tggtggggtt tttgtttgt taatttagaa caatgccatt    420
ccatttcctg tataatgagt cgcttctttg ttgtaaactc tccttagaat ttcttgggag    480
aggaactgaa cagaacattg atttcctatg tgagagaatt cttagaattt aaataaacct    540
attggttaaa ctgaaccac aaaattagca ttttactaat cagtaggttt aaatagcttg    600
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position 443 to 448

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gattagacca catataatgt aacttatttc acaaggtaaa taattataat aaataatatg    180
gattaactga gttttaaaag gtgaaataaa taatgaattc ttctcatggt cttgtatggt    240
aataaaaatt gaaaaatttt gaagacccca ttttgtccca agaatttcct ttacagggtat    300
tgaatttttc aaaggttaca aaggaaattt tattgatata ataatgcat gttctcataa    360
taaccataaa tctagggttt tggtggggtt tttttgttt gtaatttag aacaatgcca    420
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agaggaaactg aacagaacet tgatttccta tgtgagagaa ttcttagaat taaataaac    540
ctgttggtta aactgaacc acaaaattag cattttacta atcagtaggt taaatagct    600
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position 439 to 444 deletion G and TTT between 390 and 396
compaired with Allel 2

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gattaactga gttttaaaag gtgaataaaa taatgaattc ttctcatggt cttgtatggt    240
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tgaatttttc aaagggtaca aaggaaattt tattgatata ataaatgcat gttctcataa    360
taaccataaa tctaggggtt tgttgggggt ttttttgta atttagaaca atgccattcc    420
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gaactgaaca gaacattgat ttcctatgtg agagaattct tagaatttaa ataaacctgt    540
tggttaaaact gaaaccacaa aattagcatt ttactaatca gtaggtttaa atagcttgga    600
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<222> LOCATION: (1)..(650)
<223> OTHER INFORMATION: Bindingsite for transcriptionfactors: AP-1 at
position 434 to 441, ABF1 at position 469 to 483, YY-1 at
position 439 to 444; mutation (SNP) at position 480 (G to C),
developing a ABF1-bindingsite;
deletion G and TTT between position 390 and 396 compaired with Al
lel 2

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tgaatttttc aaagggtaca aaggaaattt tattgatata ataaatgcat gttctcataa    360
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gaactgaaca gaacattgat ttcctatgtg agagaattct tagaatttaa ataaacctgt    540
tggttaaaact gaaaccacaa aattagcatt ttactaatca gtaggtttaa atagcttgga    600
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<400> SEQUENCE: 8
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1. Genetic marker at the 5'-flanking region of the α S1 casein gene (CSN1S1) characterized by the fact that it contains the nucleotide sequence 1-1061, preferably the nucleotide sequence 1-655 at the 5'-flanking region of the α S1 casein gene.

2. Genetic marker according to patent claim 1 characterized by its amplification by means of PCR reaction either through

Primer 1
CSN1S1pro1f (5' GAA TGA ATG AAC TAG TTA CC 3')

Primer 2
CSN1S1pro1r (5' GAA GAA GCA GCA AGC TGG 3')

or through

Primer 1
CSN1S1pro1f (5' GAA TGA ATG AAC TAG TTA CC 3')

Primer 3
CSN1S1pro2r (5' CCT TGA AAT ATT CTA CCA G 3')

3. Genetic marker according to patent claim 1 characterized by its variability within milk breeds.

4. Genetic marker according to patent claim 1 characterized by its utilization in order to determine the allelic state at the 5'-flanking region of the α S1 casein gene.

5. Procedure to determine the allelic state of the 5'-flanking region of the α S1 casein gene, characterized by the following steps:

- a) provision of the source material of the organism to be examined
- b) isolation of the genetic material
- c) targeted isolation or enrichment of the marker fragment at the 5' region of the α S1 casein gene or of a sequence, which contains portions of the marker sequence, preferably the fragment 1 to 655 of the marker sequence out of the α S1 casein gene
- d) Proof of the allelic state in the isolated or enriched sequence fragment of the marker fragment of the α S1 casein gene.

6. Procedure according to patent claim 5 characterized by the utilization of source material coming from an animal, particularly a mammal, in particular a bovine, a sheep or a goat, including breed animals and embryos of these species.

7. Procedure according to patent claim 5 characterized by the utilization of blood, leukocytes, tissue including biopsy material, milk, sperm, hair, individual cells including cell material from embryos, a bacteria culture or isolated chromosomes as source material.

8. Procedure according to patent claim 5 characterized by the utilization of source material coming from a genetically modified organism (GMO) which contains the marker fragment of the α S1 casein gene.

9. Procedure according to patent claim 5 characterized by the utilization of genetic material containing genomic DNA or RNA from animals, plasmid DNA from bacteria, from artificial chromosomes such as BACs and YACs.

10. Procedure according to patent claim 5 characterized by achieving the enrichment of the marker segment of the α S1 casein gene by means of polymerase chain-reaction.

11. Procedure according to patent claim 5 characterized by the enrichment of the marker segment of the α S1 casein gene by means of polymerase chain-reaction with the oligonucleotides

Primer 1
CSN1S1pro1f (5' GAA TGA ATG AAC TAG TTA CC 3')

Primer 2
CSN1S1pro1r (5' GAA GAA GCA GCA AGC TGG 3')

Primer 3
CSN1S1pro2r (5' CCT TGA AAT ATT CTA CCA G 3')

as primers, whereby the following combinations are selected: primer 1 with primer 2 and primer 2 with primer 3.

12. Procedure according to patent claim 5 characterized by the determination the allelic state by means of SSCP, RFLP, OLA, TGGE, ASPCR, PCR-ELISA, microarray method or through nucleic acid sequencing.

13. Procedure according to patent claim 5 characterized by detection of one or more of the allelic states of the marker sequence of the α S1 casein gene.

14. Utilization of the procedure according to claim 5 in order to examine the animals' milk production traits, independently of age and lactation.

15. Utilization of the procedure according to claim 5 in order to select organisms which carry a certain allelic state or a certain genotype of the marker sequence of the α S1 casein gene or a portion thereof.

16. Utilization of the procedure according to claim 5 in breeding programs, particularly for a marker-supported selection.